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(54) Title: **PREVENTION OF DEVELOPMENT OF DYSKINESIAS**

(57) Abstract: The present invention relates to the prevention of the development of sensitization caused by chronic use of dopaminergic agents using an alfa2-adrenoceptor antagonist or a pharmaceutically acceptable ester or salt thereof.

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PREVENTION OF DEVELOPMENT OF DYSKINESIAS

The present invention relates to a method for preventing the development of
5 sensitization caused by chronic use of dopaminergic agents. Especially, the present
invention relates to the use of α_2 -adrenoceptor antagonists in the prevention of the
development of sensitization caused by chronic use of dopaminergic agents.

Additional objects and advantages of the invention will be set forth in part in the
description which follows, and in part will be obvious from the description, or may be
10 learned by practice of the invention.

BACKGROUND OF THE INVENTION

Dopamine is a neurotransmitter that influences on many behavioural functions such
as locomotor activity and learning and it is involved in neuropsychiatric disorders such as
15 Parkinson's Disease and schizophrenia (Beninger 1983). Stimulants like amphetamine and
cocaine enhance dopamine release in the CNS by inhibition of dopamine uptake from the
synaptic cleft. When amphetamine is administered repeatedly in daily doses, the increase
in motor activity is higher than after one single dose, a phenomenon that is called
amphetamine sensitization. This phenomenon is connected with the development of drug
20 dependency, but it may also be considered as a dyskinesia caused by chronic use of
dopaminergic agents.

In animal models of α_2 -adrenoceptor antagonists, such as idazoxan and
atipamezole, are known to have therapeutic effects on the symptoms of Parkinson's
Disease (PD). In animal models of PD, they also after acute administration potent the
25 motor responses of dopaminergic agents such as, apomorphine, L-3,4-dihydroxyphenyl-
alanine (L-dopa) and amphetamine. In addition, in PD patients and animal models where
the dyskinesias are developed after chronic administration of L-dopa, α_2 -adrenoceptor
antagonists have decreased the dyskinesias by enhancing inhibition in so called indirect
pathway of basal ganglia which is influenced by D2 dopamine receptors (Brotchie, J.M.,
30 Parkinson's Disease Advances in Neurology, Vol. 80., in Advances in Understanding the
Neural Mechanisms Underlying L-Dopa-Induced Dyskinesias, Edited by Gerald M. Stern,

Lippincott William & Wilkins, Philadelphia 1999). However, the most effective way to control dyskinesias in patients is to prevent their development during dopaminergic treatment. The development of dyskinesia has been proposed to involve the overactivity of so called direct pathway of basal ganglia which is influenced by D1 dopamine receptors.

- 5 According to the knowledge of the inventors the use of alpha2-adrenoceptor antagonist in the prevention of the development of dyskinesias has not been suggested or shown before.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the mean 2-h overall ambulatory activity counts \pm S.E.M. after six
 10 repeated administrations of D-amphetamine 2 mg/kg s.c. and the effect of atipamezole 1 mg/kg s.c. pre-treatment 20 minutes before D-amphetamine challenge, n= 20-72. Groups: saline (days 1-8 saline); saline-amphetamine (days 1-7 saline and day 8 D-amphetamine); amphetamine (days 1-8 D-amphetamine); atipamezole (days 1-8 atipamezole before saline); atipamezole-amphetamine (days 1-8 atipamezole before D-
 15 amphetamine). Statistical significances: locomotor activity of the group compared to saline-saline-group (***P<0.001, **P<0.01 and *P<0.05) and locomotor activity of the group compared to amphetamine-amphetamine-group (⁺⁺⁺P<0.001, ⁺⁺P<0.01 and ⁺P<0.05).

Figure 2 shows the mean 2-h overall ambulatory activity counts \pm S.E.M. at day 9,
 20 n= 5-29. Chronic treatment groups: saline (days 1-8 saline); atipam. (days 1-8 atipamezole 1 mg/kg); amph. (days 1-8 D-amphetamine 2 mg/kg); atipam,- amph. (days 1-8 atipamezole 1 mg/kg 20 minutes before D-amphetamine 2 mg/kg). All drugs were administrated subcutaneously in volume 0,1 ml. Drug treatments at day 9: saline (saline 20 min before saline); 1 mg/kg atipam. (atipamezole 1 mg/kg 20 min before saline); 2 mg/kg
 25 amph. (saline 20 min before D.-amphetamine 2 mg/kg); 0,3 mg/kg atipam.- 2 mg/kg amph. (atipamezole 0,3 mg/kg 20 min before D-amphetamine 2 mg/kg); 1 mg/kg atipam.- 2 mg/kg amph. (atipamezole 1 mg/kg 20 min before D-amphetamine 2 mg/kg); Statistical significances: locomotor activity of the group compared to saline-saline-group (***P<0.001, **P<0.01 and *P<0.05), locomotor activity of the group compared to amph.-
 30 2 mg/kg amph.-group (⁺⁺⁺P<0.001, ⁺⁺P<0.01 and ⁺P<0.05) and locomotor activity of the group compared to the chronic saline group with same drug treatment at day 9

($^{\circ\circ}P < 0.001$, $^{\circ}P < 0.01$ and $^{\circ}P < 0.05$).

DETAILED DESCRIPTION OF THE INVENTION

Applicants have surprisingly discovered that an alfa2-adrenoceptor antagonist, atipamezole, reduced the development and expression of sensitization (motor overactivity) when given chronically in combination with a dopaminergic stimulator, D-amphetamine, in mice. Thus, alfa2-adrenoceptor antagonists such as atipamezole, and their pharmacologically acceptable esters or salts, can be used for prevention of development of sensitizational conditions caused by chronic use of dopaminergic agents. The sensitizational conditions include e.g., dyskinesias and psychosis developed by chronic use of dopaminergic agents such as, apomorfine, amphetamine, and L-dopa.

Nigrostriatal dopaminergic neurons from substantia nigra to the dorsal striatum are believed to be central in the modulation of extrapyramidal motor processes. This circuitry is disturbed in PD and cause symptoms typical to PD like tremor, rigidity and difficulties in the initiation of motor actions. L-dopa has been used to relieve symptoms of PD. However, many complications are observed after continuous treatment with L-dopa, of which the most common are abnormal involuntary movements called dyskinesia (Barbeau 1974). The plastic changes in dopaminergic system controlling motor responses are thought to be responsible for development of dyskinesia. Alfa2-adrenoceptor antagonists, such as atipamezole are found to enhance neuronal plasticity (Puurunen K, Jolkkonen J, Sirviö J, Haapalinna A, Sivenius J. An alpha-2 adrenergic antagonist, atipamezole, facilitates behavioral recovery after focal cerebral ischemia in rats. *Neuropharmacology* 40: 597-606, 2001). Furthermore, the activation of D1 dopamine receptors and the blockade of alpha-2 adrenoceptors can cause the same kind of effect in the second messenger systems of basal ganglia. Thus, repeated administration of alfa2-adrenoceptor antagonist might be inactive or even enhance the development of dyskinesias. Locomotor hyperactivity caused by chronic activation of dopaminergic transmission by amphetamine is also a dysfunction in motor activity and is also due to sensitization effect like dyskinesia seen after chronic L-dopa treatment.

The present invention provides a new solution in the pharmacotherapy of Parkinson's disease with alfa2-adrenoceptor antagonist by preventing the development of dyskinesia caused by chronic use of dopaminergic agents.

Alfa2-adrenoceptor antagonist of the invention include, without limitation, 5 atipamezole, idazoxan, efaroxan and their analogs and pharmaceutically acceptable salts. 4-(2-ethyl-2,3-dihydro-1H-inden-2-yl)-1H-imidazole, known as atipamezole, and its pharmaceutically acceptable acid addition salts with inorganic and organic acids generally used for the purpose, are described in U.S. Patent. No. 4,689,339. The halogenated analogs of atipamezole, for example 4-(2-ethyl-5-fluoro-2,3-dihydro-1H-inden-2-yl)-1H-imidazole 10 and 4-(2-ethyl-5,6-difluoro-2,3-dihydro-1H-inden-2-yl)-1H-imidazole and their pharmaceutically acceptable acid addition salts have been described in U.S. Patent No. 5,498,623. Idazoxan, 2-(2-(1,4-benzodioxanyl))-2-imidazoline, and efaroxan, 2-(2-ethyl-2,3-dihydro-2-benzofuranyl)-4,5-dihydro-1H-imidazole and their pharmaceutically acceptable acid addition salts, are described in U.S. Patents Nos. 4,818,764 and 4,411,908, 15 respectively.

To achieve optimal results, the treatment with the alfa-2 antagonist is preferably started at the same time as the treatment with the dopaminergic agent. The precise amount of the drug to be administered to a mammal according to the present invention is dependent on numerous factors known to one skilled in the art, such as, the compound to be 20 administered, the general condition of the patient, the condition to be treated, the desired duration of use, the type of mammal, the method and route of administration etc. For example, for atipamezole given together with L-dopa, the usual daily dosage will be from 1 to 50 mg, preferably from 10 to 30 mg, divided in 1 to 4 individual doses. Thus, the most preferable single dose for atipamezole will be 10 mg. The alfa-2 antagonist is preferably 25 given simultaneously with the dopaminergic agent.

Typical routes of administration include, without limitation, oral, transdermal, transmucosal, and parenteral routes.

The invention will be further clarified by the following example, which is intended 30 to be purely exemplary of the invention.

EXAMPLE 1

The effects of atipamezole on the locomotor hyperactivity induced by repeated administration of D-amphetamine were studied in male mice.

Animals

- 5 Experiments were performed with C57BL/6J strain male mice from Jackson Laboratories. Mice were transferred to laboratory at least 2 weeks prior to use. The mice were from 8 to 20 weeks of age at the beginning of an experiment. Groups of 10 mice were housed in standard polypropylene cages (38 X 22 X 15 cm) with free access to standard certified pelleted food (RM1 Maintenance Expanded SQC; Special Diet Services, Essex, 10 UK) and water. Ambient temperature was 22 ± 1 C°, and a 12:12 h light/dark cycle was maintained with lights on at 6 A.M. All experiments were carried out between 7 A.M. and 5 P.M. The animal care was performed in accordance with International Council for Laboratory Animal Science (ICLAS) guidelines.

Drugs

- 15 D-Amphetamine sulphate (Sigma, St. Louis, MO, U.S.A.) and atipamezole HCl (Orion Corporation, Orion Pharma, Turku, Finland) were dissolved in saline (0.9% NaCl) and administered subcutaneously (s.c.) in a 5 ml/kg volume.

Motor Activity Testing

- 20 The locomotor activity of the mice was measured in transparent standard polypropylene animal cages (38 X 22 X 15 cm) with transparent cover and aspen bedding on the floor. Test cages were placed middle of the photobeam frame system (Photobeam Activity System PAS, Cage Rack, San Diego Instruments, San Diego, CA). Computer control unit registered the interruptions of photobeams from 16 individual cages. Three 25 different types of movements were monitored: 1) ambulations (large horizontal movements), 2) fine movements (smaller horizontal movements) and 3) rearings (vertical movements). Locomotor activity was measured at 5-min intervals for 2 h immediately after D-amphetamine or saline administrations.

Sensitization schedule and atipamezole treatment

D-amphetamine was administered subcutaneously (s.c.) at the dose of 2 mg/kg. Atipamezole was administered s.c. at the dose of 1 mg/kg 20 min before locomotor activity measurement.

In the chronic treatment group mice were administrated during eight days to elicit provoked locomotor hyperactivity to D-amphetamine and the effect of the atipamezole to the locomotor activity. Mice groups in the chronic treatment schedule were saline, saline-amphetamine, amphetamine, atipamezole and atipamezole-amphetamine. A day before experiment, mice were habituated to the test environment. Test groups with different drug treatments were administrated during four consecutive days. At days five and six there were no drug administrations and motor activity testing. At days seven and eight, the produced locomotor hyperactivity and effect of a single exposure of D-amphetamine (saline-amphetamine -group) were analysed. (Table 1).

Table 1
Chronic treatment

Time	saline	saline-amphetamine	amphetamine	atipamezole	atipamezole-amphetamine
Habituation	saline	saline	saline	saline	saline
Day 1	saline	saline	amph.	atipam.	atipam. and amph.
Day 2	saline	saline	amph.	atipam.	atipam. and amph.
Day 3	saline	saline	amph.	atipam.	atipam. and amph.
Day 4	saline	saline	amph.	atipam.	atipam. and amph.
Day 5	no injection	no injection	no injection	no injection	no injection
Day 6	no injection	no injection	no injection	no injection	no injection
Day 7	saline	saline	amph.	atipam.	atipam. and amph.
Day 8	saline	amph.	amph.	atipam.	atipam. and amph.

15

At day nine, the effect of different atipamezole and amphetamine administrations to the locomotor activity on the chronic treatment groups were analysed. Used treatments were saline, 1 mg/kg atipamezole, 2 mg/kg D-amphetamine, 0,3 mg/kg atipamezole- 2 mg/kg D-amphetamine and 1 mg/kg atipamezole- 2 mg/kg D-amphetamine. Chronic treatment groups were saline-, atipamezole-, amphetamine- and atipamezole-amphetamine groups. Chronic groups were treated following schedule in Table 2.

20

Table 2
Drug treatments at day 9

Drug treatment	Chronic group			
	saline	atipamezole	amphetamine	atipamezole- amphetamine
saline	Yes	No	No	No
1 mg/kg atipamezole	Yes	Yes	No	No
2 mg/kg amphetamine	Yes	Yes	Yes	Yes
0,3 mg/kg atipamezole- 2 mg/kg amphetamine	Yes	No	Yes	No
1 mg/kg atipamezole- 2 mg/kg amphetamine	Yes	No	Yes	Yes

All data are presented as mean \pm SEM. Statistical analysis were performed using SPSS 9.0 for Windows (SPSS, Chicago, IL). Separate repeated measures analyses of variance (ANOVA) were performed on each variable for each experiment grouped on time and treatment group. Results were analysed separately, because data were collected in separate experiments with different study design. When significance ($P < 0.05$) between treatment groups were found comparisons at each time point (date or min) were analyzed by using LSD post-hoc test.

10 RESULTS

Locomotor activity

Effect of repeated administration of D-amphetamine and atipamezole in chronic treatment groups

Figure 1 illustrates the development of behavioural sensitization after six repeated administrations of D-amphetamine (2 mg/kg) and the effect of atipamezole (1 mg/kg) pre-treatment 20 minutes before D-amphetamine challenge in mice. There was a significant difference between the chronic treatment groups [$F(1,184)=1618.9$, $P < 0.001$]. The activity counts were dependent on the administration Day [$F(6,1104) = 107.7$, $P < 0.001$] and there was a significant interaction between Day X Group [$F(24,1104) = 53.2$, $P < 0.001$]. Mice treated with D-amphetamine of six consecutive days (group amphetamine-amphetamine) showed a progressive enhance in ambulatory activity compared to saline group. At Day eight, mice from group saline-amphetamine were also administered with D-amphetamine, but there was still a significant difference compared group amphetamine to groups saline and saline-amphetamine ($P < 0.001$).

Atipamezole pre-treatment before saline challenge tended to enhance locomotor activity after daily atipamezole for six days, but there was not statistical significant difference between the groups atipamezole-saline and saline at Day eight ($P=0.43$).

Atipamezole pre-treatment before D-amphetamine administration over the course of exposure decreased significantly D-amphetamine-induced locomotion in mice. There was statistical significant difference between groups amphetamine-amphetamine and atipamezole-amphetamine at Day 2 ($P<0.05$) and Days 3-8 ($P<0.001$). This indicates that atipamezole pre-treatment prevented D-amphetamine-induced locomotor hyperactivity over the course of the exposure.

10 Effect of the different drug treatments at Day 9

Figure 2 illustrates the effect of the different drug treatments in chronic treatment groups at Day 9. Atipamezole pre-treatment alone did not alter total ambulatory activity counts in the saline group ($P=0.81$). D-amphetamine significantly enhanced locomotor activity in the saline group ($P<0.001$), but the activity was not same level compared to amphetamine group ($P<0.001$). Atipamezole (0.3 mg/kg) pre-treatment 20 minutes before D-amphetamine challenge amplified locomotor activity ($P=0.016$), but with dosage 1 mg/kg had no effect compared D-amphetamine alone ($P=0.364$). (Figure 2, Group saline).

Atipamezole (1 mg/kg) pre-treatment over the course of exposure had tendency to elevate locomotor activity before saline and D-amphetamine challenge, but elevation was not statistically significant (Figure 2, Group atipam.).

Mice in Group amphetamine developed strong ambulatory sensitization during repeated administration of D-amphetamine (2 mg/kg) ($P<0.001$, Figure 2). Atipamezole doses (0.3 and 1 mg/kg) both clearly attenuated the D-amphetamine-induced locomotor hyperactivity (0.3 mg/kg $P=0.005$; 1 mg/kg $P<0.001$).

Atipamezole pre-treatment (1 mg/kg) 20 min before D-amphetamine administration over the course of exposure (Group atipamezole-amphetamine) decreased significantly D-amphetamine induced locomotion. There was statistical significant difference between groups amphetamine-amphetamine and atipamezole-amphetamine also at Day 9 ($P<0.001$). Part of mice from Group atipamezole-amphetamine were administrated only with D-amphetamine (2 mg/kg) at Day 9 and there was still statistical significant difference compared to amphetamine-amphetamine -group ($P=0.026$). This latter result is important as it shows that

atipamezole causes a true reduction in the development of sensitization not just the suppression of this expression.

Those skilled in the art will recognize that while specific embodiments have been illustrated and described, various modifications and changes may be made without
5 departing from the spirit and scope of the invention.

Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

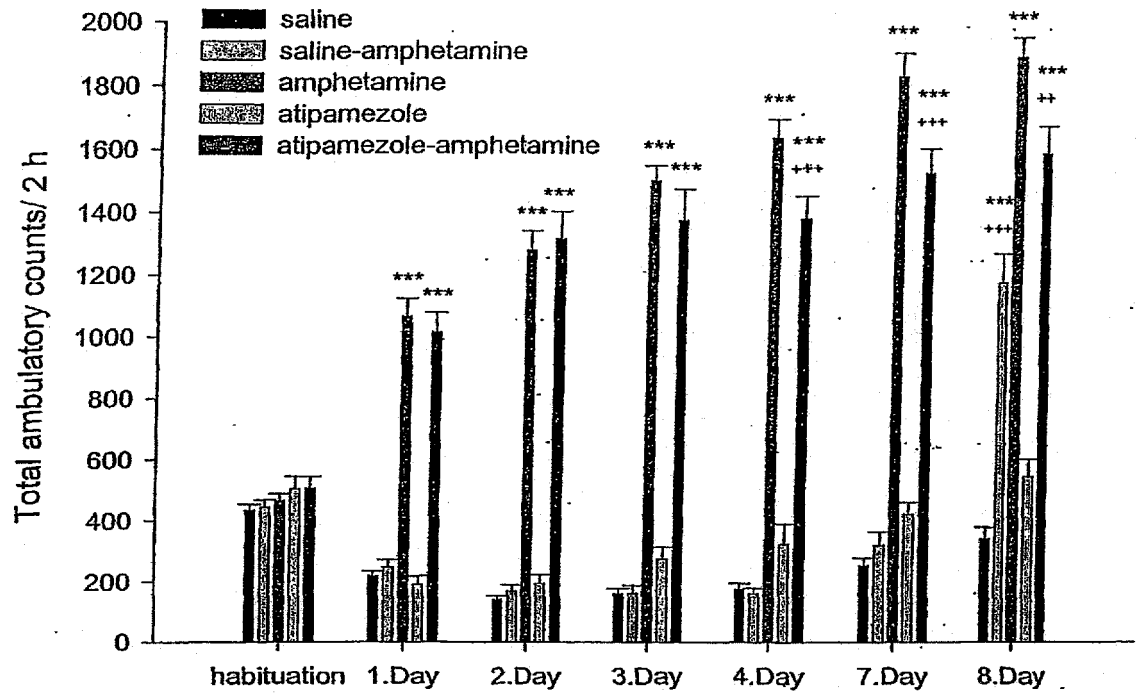
10 The references discussed herein are specifically incorporated by reference in their entirety.

CLAIMS:

1. A use of an alfa2-adrenoceptor antagonist in the manufacture of a medicament for the prevention of the development of sensitization caused by chronic use of dopaminergic agents.
5
2. The method according to claim 1 wherein the sensitization is dyskinesia seen in Parkinson's Disease after chronic treatment of L-dopa.
- 10 3. The method according to any one of claims 1-2, wherein the alfa2-adrenoceptor antagonist is atipamezole or a pharmaceutically acceptable salt thereof.
4. The method according to any one of claims 1-2, wherein the alfa2-adrenoceptor antagonist is idazoxan or a pharmaceutically acceptable salt thereof.
15
5. The method according to any one of claims 1-2, wherein the alfa2-adrenoceptor antagonist is efaroxan or a pharmaceutically acceptable salt thereof.
6. The method according to any one of claims 1-2, wherein the alfa2-adrenoceptor antagonist is example 4-(2-ethyl-5-fluoro-2,3-dihydro-1H-inden-2-yl)-1H-imidazole or a
20 pharmaceutically acceptable salt thereof.

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Figure 1



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Figure 2

